

Applicant : Donald W. Landry  
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In the specification:

Please replace the paragraph beginning on page 2, line 11, with the following replacement paragraph:

B1

The first catalytic antibodies to degrade cocaine, Mab 3B9 and Mab 6A12, were elicited by an immunogenic conjugate (TSA 1) of a phosphonate monoester transition-state analog (9). The rate acceleration of these first artificial cocaine esterases ( $10^2$ - $10^3$ ) corresponded in magnitude to their relative stabilization of the ground-state to the transition-state ( $\sim K_m/K_i$ ). Catalytic antibodies with more potent catalytic mechanisms and with higher turnover rates are possible and, it has been estimated, necessary for clinical applications. Increased activity can be pursued either through repeated hybridoma generation or through mutagenesis of catalytic antibodies in hand. However, sequencing of the variable domains of Mab's 3B9 and 6A12 revealed 93% homology at the complementarity determining regions (see below). Such a lack of diversity has been noted previously for catalytic antibodies (10) and limits the opportunities for improving activity since a particular class of homologous catalytic antibodies may fail to optimize to the desired activity. A potential solution to this problem, that would not compromise the core structure of the analog, would be to vary the surfaces of the analog rendered inaccessible by attachment to carrier protein and thereby present distinct epitopes for immunorecognition.

Please replace the paragraph beginning on page 4, line 28, with the following replacement paragraph:

B2

The invention provides a catalytic antibody capable of

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degrading cocaine comprising (i) a light chain, wherein the amino acid sequence of complementarity determining region 1 is RSSXGTITXXNYAN (Seq ID No: 73), the amino acid sequence of complementarity determining region 2 is XNNYRPP (Seq ID No: 74) and the amino acid sequence of complementarity determining region 3 is ALWYSNHWV (Seq ID No: 75) and (ii) a heavy chain, wherein the amino acid sequence of complementarity determining region 1 is DYNMY (Seq ID No: 76), the amino acid sequence of complementarity determining region 2 is YIDPXNGXXFYFNQKFXG (Seq ID No: 77) and the amino acid sequence of complementarity determining region 3 is GGGLFAX (Seq ID No: 78), wherein X can be any amino acid.

*B2*

Please replace the paragraph beginning on page 5, line 36, with the following replacement paragraph:

The present invention provides a catalytic antibody capable of degrading cocaine comprising (i) a light chain, wherein the amino acid sequence of complementarity determining region 1 is KSSQSLLYSDGKTYLN (Seq ID No: 43), the amino acid sequence of complementarity determining region 2 is LVSKLDS (Seq ID No: 44) and the amino acid sequence of complementarity determining region 3 is VQGYTFPLT (Seq ID No: 45) and (ii) a heavy chain, wherein the amino acid sequence of complementarity determining region 1 is DHWMH (Seq ID No: 70), the amino acid sequence of complementarity determining region 2 is TIDLSDTYTGYNQNFKG (Seq ID No: 71) and the amino acid sequence of complementarity determining region 3 is RGFDY (Seq ID No: 72).

*B3*

Please replace the paragraph beginning on page 6 line 14, with the following replacement paragraph:

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In another embodiment, the present invention provides a polypeptide comprising a light chain domain with complementarity determining region 1 having amino acid sequence RSSXGTITXXNYAN (Seq ID No: 73), complementarity determining region 2 having amino acid sequence XNNYRPP (Seq ID No: 74) and complementarity determining region 3 having amino acid sequence ALWYSNHWV (Seq ID No: 75) interposed between appropriate framework regions, said light chain domain being linked to a heavy chain domain with complementarity determining region 1 having amino acid sequence DYNMY (Seq ID No: 76), complementarity determining region 2 having amino acid sequence YIDPXNGXIFYNQKFXG (Seq ID No: 77) and complementarity determining region 3 having amino acid sequence GGGLFAX (Seq ID No: 78) interposed between appropriate framework regions such that said polypeptide assumes a conformation suitable for degrading cocaine.

Please replace the paragraph beginning on page 7, line 31, with the following replacement paragraph:

In another embodiment, the invention provides a polypeptide comprising a light chain domain with complementarity determining region 1 having amino acid sequence KSSQSLLYSDGKTYLN (Seq ID No: 43), complementarity determining region 2 having amino acid sequence LVSKLDS (Seq ID No: 44) and complementarity determining region 3 having amino acid sequence VQGYTFPLT (Seq ID No: 45) interposed between appropriate framework regions, said light chain domain being linked to a heavy chain domain with complementarity determining region 1 having amino acid sequence DHWMH (Seq ID No: 70), complementarity determining region 2 having amino acid sequence TIDLSDTYTGYNQNFKG (Seq

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ID No: 71) and complementarity determining region 3 having amino acid sequence RGFDY (Seq ID No: 72) interposed between appropriate framework regions such that the polypeptide assumes a conformation suitable for degrading cocaine.

Please replace the paragraph beginning on page 16, line 3, with the following replacement paragraph:

B6  
The invention provides a catalytic antibody capable of degrading cocaine comprising (i) a light chain, wherein the amino acid sequence of complementarity determining region 1 is RSSXGTITXXNYAN (Seq ID No: 73), the amino acid sequence of complementarity determining region 2 is XNNYRPP (Seq ID No: 74) and the amino acid sequence of complementarity determining region 3 is ALWYSNHWV (Seq ID No: 75) and (ii) a heavy chain, wherein the amino acid sequence of complementarity determining region 1 is DYNMY (Seq ID No: 76), the amino acid sequence of complementarity determining region 2 is YIDPXNGXXFYNQKFXG (Seq ID No: 77) and the amino acid sequence of complementarity determining region 3 is GGGLFAX (Seq ID No: 78).

Please replace the paragraph beginning on page 17 line 11, with the following replacement paragraph:

B7  
The present invention provides a catalytic antibody capable of degrading cocaine comprising a light chain wherein the amino acid sequence of complementarity determining region 1 is KSSQSLLYSDGKTYLN (Seq ID No: 43), the amino acid sequence of complementarity determining region 2 is LVSKLDS (Seq ID No: 44) and the amino acid sequence of complementarity determining region 3 is VQGYTFPLT (Seq ID

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No: 45) and a heavy chain wherein the amino acid sequence of complementarity determining region 1 is DHWMH (Seq ID No: 70), the amino acid sequence of complementarity determining region 2 is TIDLSDTYTGYNQNFKG (Seq ID No: 71) and the amino acid sequence of complementarity determining region 3 is RGFDY (Seq ID No: 72).

Please replace the paragraph on page 49, lines 10-12, with the following replacement paragraph:

Table 3. Deduced amino acid sequences of catalytic antibodies light chain CDR's (Panel A) (SEQ ID Nos: 19-45) and heavy chain CDR's (Panel B) (SEQ ID Nos: 46-72).

Please delete the Brief Description of the Figures, beginning on page 10, line 1, and replace it with the following:

Brief Description of the Figures

**Figure 1.** Hydrolysis of the benzoyl ester of cocaine. Presumed tetrahedral intermediate formed along the reaction pathway is shown. General structure of a phosphonate monoester analogs of the benzoyl ester: TSA 1, TSA 2, TSA 3. TSA 4.

**Figure 2.** Synthesis of TSA-1.

**Figure 3.** Synthesis of TSA-2.

**Figure 4.** Synthesis of TSA-3.

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**Figure 5.** Plot of log  $(K_m/K_{TSA_4})$  versus log  $(k_{cat}/k_{uncat})$  for catalytic antibodies generated by TSA<sub>1</sub>, 2, and 3. Data represented in this figure are from Tables 1 and 2. Linear relationship by least squares method; r=0.85 excluding Mab 15A10 and 8G4G.

**Figure 6.** Alignment of amino acid sequences of Lambda light chains, wherein

9A(lam9)vari (SEQ ID No: 1) indicates the amino acid sequence of the variable domain of the Lambda light chain of the antibody 9A3;

19G(lam5)vari (SEQ ID No: 2) indicates the amino acid sequence of the variable domain of the Lambda light chain of the antibody 19G8;

15A10L vari (SEQ ID No: 3) indicates amino acid sequence of the variable domain of the Lambda light chain of the antibody 15A10;

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G7(lam4) vari (SEQ ID No: 4) indicates the amino acid sequence of the variable domain of the Lambda light chain of the antibody 8G4G;

**Figure 7.** Alignment of amino acid sequences of Kappa light chains, wherein

3B9 K vari (SEQ ID No: 5) indicates the amino acid sequence of the variable domain of the Kappa light chain of the antibody 3B9;

6A12 K vari (SEQ ID No: 6) indicates the amino acid sequence of the variable domain of the Kappa light chain of the antibody 6A12;

12H(L2)k vari (SEQ ID No: 7) indicates the amino acid sequence of the variable domain of the

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2A k vari Kappa light chain of the antibody 12H1;  
E2(L7) k Vari (SEQ ID No: 8) indicates the amino acid  
sequence of the variable domain of the  
Kappa light chain of the antibody 2A10;  
Kappa light chain of the antibody 8G4E.

**Figure 8.** Alignment of amino acid sequence of heavy chains,

wherein

3B9 vari (SEQ ID No: 10) indicates the amino acid sequence of the variable domain of the heavy chain of the antibody 3B9;  
6A12 heavy (SEQ ID No: 11) indicates the amino acid sequence of the variable domain of the heavy chain of the antibody 6A12;  
12H H vari (SEQ ID No: 12) indicates the amino acid sequence of the variable domain of the heavy chain of the antibody 12H1;  
2AH-3 (SEQ ID No: 13) indicates the amino acid sequence of the variable domain of the heavy chain of the antibody 2A10;  
9(H-3)vari (SEQ ID No: 14) indicates the amino acid sequence of the variable domain of the heavy chain of the antibody 9A3;  
19h6-3 vari (SEQ ID No: 15) indicates the amino acid sequence of the variable domain of the heavy chain of the antibody 19G8;  
15A10 Vari (SEQ ID No: 16) indicates amino acid sequence of the variable domain of the heavy chain of the antibody 15A10;  
E2(H8) Vari (SEQ ID No: 17) indicates the amino acid sequence of the variable domain of the heavy chain of the antibody 8G4E.  
G7(H8) vari (SEQ ID No: 18) indicates the amino

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acid sequence of the variable domain of  
the heavy chain of the antibody 8G4G;

**Figure 9.** Nucleotide sequence of the light chain of the anti-cocaine catalytic antibody 15A10 (SEQ ID No: 120). The amino acid sequence is set forth in (SEQ ID No: 121).

**Figure 10.** Nucleotide sequence of the heavy chain of the anti-cocaine catalytic antibody 15A10 (SEQ ID No: 85). The amino acid sequence is set forth in (SEQ ID No: 86).

**Figure 11.** Nucleotide sequence of the light chain of the anti-cocaine catalytic antibody 19G8 (SEQ ID No: 87). The amino acid sequence is set forth in (SEQ ID No: 88).

**Figure 12.** Nucleotide sequence of the heavy chain of the anti-cocaine catalytic antibody 19G8 (SEQ ID No: 89). The amino acid sequence is set forth in (SEQ ID No: 90).

**Figure 13.** Nucleotide sequence of the light chain of the anti-cocaine catalytic antibody 9A3 (SEQ ID No: 91). The amino acid sequence is set forth in (SEQ ID No: 92).

**Figure 14.** Nucleotide sequence of the heavy chain of the anti-cocaine catalytic antibody 9A3 (SEQ ID No: 93). The amino acid sequence is set forth in (SEQ ID No: 94).

**Figure 15.** Nucleotide sequence of the light chain of the anti-cocaine catalytic antibody 8G4G (SEQ ID No: 95). The amino acid sequence is set forth in (SEQ ID No: 96).

**Figure 16.** Nucleotide sequence of the heavy chain of the anti-cocaine catalytic antibody 8G4G (SEQ ID No: 97). The

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amino acid sequence is set forth in (SEQ ID No: 98).

**Figure 17.** Nucleotide sequence of the light chain of the anti-cocaine catalytic antibody 3B9 (SEQ ID No: 99). The amino acid sequence is set forth in (SEQ ID No: 100).

**Figure 18.** Nucleotide sequence of the heavy chain of the anti-cocaine catalytic antibody 3B9 (SEQ ID No: 101). The amino acid sequence is set forth in (SEQ ID No: 102).

**Figure 19.** Nucleotide sequence of the light chain of the anti-cocaine catalytic antibody 6A12 (SEQ ID No: 103). The amino acid sequence is set forth in (SEQ ID No: 104).

**Figure 20.** Nucleotide sequence of the heavy chain of the anti-cocaine catalytic antibody 6A12 (SEQ ID No: 105). The amino acid sequence is set forth in (SEQ ID No: 106).

**Figure 21.** Nucleotide sequence of the light chain of the anti-cocaine catalytic antibody 2A10 (SEQ ID No: 107). The amino acid sequence is set forth in (SEQ ID No: 108).

**Figure 22.** Nucleotide sequence of the heavy chain of the anti-cocaine catalytic antibody 2A10 (SEQ ID No: 109). The amino acid sequence is set forth in (SEQ ID No: 110).

**Figure 23.** Nucleotide sequence of the heavy chain of the anti-cocaine catalytic antibody 12H1 (SEQ ID No: 111). The amino acid sequence is set forth in (SEQ ID No: 112).

**Figure 24.** Nucleotide sequence of the heavy chain of the anti-cocaine catalytic antibody 12H1 (SEQ ID No: 113). The amino acid sequence is set forth in (SEQ ID No: 114).

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**Figure 25.** Nucleotide sequence of the light chain of the anti-cocaine catalytic antibody 8G4E (SEQ ID No: 115). The amino acid sequence is set forth in (SEQ ID No: 116).

**Figure 26.** Nucleotide sequence of the heavy chain of the anti-cocaine catalytic antibody 8G4E (SEQ ID No: 117). The amino acid sequence is set forth in (SEQ ID No: 118).

**Figure 27.** The scFv of 3B9 catalytic monoclonal antibody (SEQ ID No: 119). H1 indicates the complementarity determining region 1 of the heavy chain of the antibody 3B9; H2 indicates the complementarity determining region 2 of the heavy chain of the antibody 3B9; H3 indicates the complementarity determining region 3 of the heavy chain of the antibody 3B9; L1 indicates the complementarity determining region 1 of the light chain of the antibody 3B9; L2 indicates the complementarity determining region 2 of the light chain of the antibody 3B9; L3 indicates the complementarity determining region 3 of the light chain of the antibody 3B9; FLAG indicates an epitope recognized by a known antibody; 6 x His is capable of binding to the metal Nickle; both of the Flag and 6 x His are useful for purifying the scFv..  
*B9*

**Figures 28A and 28B.**

(A) Hydrolysis of cocaine at the benzoyl ester and at the methyl ester.

(B) Presumed tetrahedral intermediate of benzoyl

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ester hydrolysis and corresponding phosphonate monoester analog.

**Figure 29.** Log dose-response relationship for Mab 15A10 on survival after LD<sub>50</sub> cocaine. Male rats received intravenous saline (n=8), or Mab 15A10 at 5 mg/kg (n=5), 15 mg/kg (n=5) or 50 mg/kg (n=5) in total volume 5 ml over 5 min. After 5 min, all animals received an intravenous catecholamine infusion as described<sup>18</sup> and an infusion of cocaine (16 mg/kg) at a rate of 1 mg/kg/min. "Survivors" completed the infusion without cardiopulmonary arrest and were observed for one hour after infusion. The effect of Mab 15A10 on survival was significant by X-square test ( $p<0.001$ ).

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**Figures 30A-30D.**

Saturation of Mab 15A10 with cocaine.

(A and B) Mean cocaine dose at seizure (A) and at death (B).

(C and D) Plasma concentration of ecgonine methyl ester (EME) (C) and cocaine at death (D). To rats prepared as in Figure 2, saline (n=17) or Mab 15A10 100 mg/kg (n=4) or Mab 1C1 100 mg/kg (n=4) in a total volume of 5 ml was administered intravenously over 5 min. Cocaine was infused intravenously at a rate of 1 mg/kg/min until cardiopulmonary arrest. Arterial plasma samples were obtained at death for determination of ecgonine methyl ester and cocaine concentrations. The significance of differences between groups, as described in the text, was determined by Wilcoxon's Rank Sign test with Bonferroni's correction for multiple comparisons.